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Distribution of Eight Vitamin E Homologs Found in 81 Plants Using LC-MS3

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Abstract

To the best of our knowledge, this is the first study to use liquid chromatography/multistage mass spectrometry to demonstrate the distribution of eight vitamin E homologs in plants. Many tocopherol homologs, which showed higher antioxidant activity than tocotrienol homologs, were discovered and α-tocopherol, which had the highest antioxidant activity among these homologs, was widely distributed in all plants. In addition, α-tocopherol occurred at high concentrations in the leaves of plants belonging to the sumac family, which is native to tropical regions. Furthermore, 25.9% of plants contained α-tocopherol alone, whereas the remaining 74.1% contained α-tocopherol in combination with other homologs. The detection frequency was the highest for a combination of α-tocopherol and γ-tocopherol, which is a precursor of α-tocopherol in plants. The highest number of vitamin E homologs was found in leaves, followed by stems, flowers, branches, and buds. Furthermore, tocotrienol homologs were present only in leaves. This indicates that the distribution of homologs in plants reflects the intensity of the antioxidant activity of homologs. It also suggests that the distribution of α-tocopherol in combination with γ-tocopherol is influenced by the α-tocopherol synthetic pathway in plants.

Keywords: Antioxidant activity; LC-MS3; Tocopherol homologs; Tocotrienol homologs; Vitamin E

Introduction

Based on its chemical structure, vitamin E can be divided into tocopherol and tocotrienol homologs. Because each homolog comprises α, β, γ, and δ forms, vitamin E has a total of eight homologs Figure 1 [1]. Herbs contain only α- and γ-tocopherol homologs [2]. Eight vitamin E homologs have been found in rice bran [3], seeds, endosperm [4], barley, wheat, and rice [5] but not in leaves and stem of plants. Because these eight homologs were previously analyzed using high-performance liquid chromatography (HPLC) with a fluorescence detector, which has a low detection sensitivity (156 ng/mL) [5], the precise distribution of homologs in plants was not well understood. We previously developed and reported regarding a highly sensitive analytical method (detection sensitivity of 20 ng/mL) that could simultaneously analyze the eight homologs present in plants using liquid chromatography/multistage mass spectrometry (LC-MS3) [6]. This LC-MS3 method made it possible to detect homologs without the effect of the matrix; therefore, a highly sensitive analysis was established, where MS3 enables extraction from plants using only methanol. β and γ homologs could not be separated using RP-HPLC with an ODS column [7]. Because these homologs could be separated under RP-HPLC conditions using long-chain alkyl-bonded C30 silica, it was not a rapid method for separating the eight components of vitamin E [7-9]. However, using LC-MS3, complete separation of these compounds under the condition used during RP-HPLC led to establishing a simultaneous and rapid determination method. In this study, we used LC-MS3 for analyzing the vitamin E homologs present in plants, and to the best of our knowledge, this is the first study to elucidate the distribution of the eight homologs in plants.

Materials and Methods

Plant materials

Of 81 plants analyzed, 44, 31, and six plants were obtained from the Botanical Garden at Showa Pharmaceutical University, the Research Institute of Evolutionary Biology, and the Tokyo University of Agriculture, respectively. The plants were then rinsed with tap water and freeze dried immediately by storing them at −80°C for 24 h.

Sample preparation

After freeze-drying, each plant part was separated and crushed using a mortar and pestle. Plastic centrifuge tubes that contained 0.2 g of each sample and approximately 2 mL of methanol (analytical grade reagent; Wako Junyaku Co., Osaka, Japan) were vortexed, and the material was extracted overnight at room temperature. The tubes were then centrifuged, and each extract was transferred to a separate tube, after which 2 mL of methanol was added to the residue in the initial centrifuge tube. This procedure was repeated twice. The extracts collected from the three extraction procedures were combined, and the volumes were adjusted to 6 mL to prepare the sample solutions.

Chemicals and reagents

In brief, 100 mL of methanol solutions, with each solution containing exactly 0.2 g of α-, β-, γ-, or δ-tocopherol (purity of all standards was>98.5%) or α-, β-, γ-, or δ-tocotrienol (purity of all standards was >98.5%), was prepared such that the standards contained 2000 μg/mL of each reagent. Each standard was diluted with methanol (LC/MS grade reagent; Wako Junyaku Co., Osaka, Japan) to prepare 20, 100, 200, 500, and 1000 ng/mL of the standard solution to produce a standard curve.

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Analytical equipment and conditions

A mass spectrometer (QTRAP5500 by AB SCIEX, Foster City, CA, USA) and an HPLC system (1200 series with a binary pump, degasser, auto sampler, and column oven by Agilent, Santa Clara, CA, USA) were used. Measurement conditions for mass spectrometer and HPLC analyses were set using those reported by Inoue et al. [6].

Analyses

A qualitative analysis was conducted by comparing the retention time and spectrum pattern of chromatograms obtained using LC-MS3. The quantity of each homolog in the sample solution was obtained from the peak area of the chromatogram of each homolog and was compared to the standard curve of each homolog. In cases wherein the homolog concentration in the sample solution was above the upper limit of the standard curve, the sample solution was appropriately diluted with methanol (LC/MS grade; Wako Junyaku Co., Osaka, Japan) for quantitation using the standard curve method.

Results

Distribution of vitamin E homologs in plants

The detection rates of the eight vitamin E homologs present in the 81 plants are shown in Figure 2. The detection rates of the tocopherol homologs were higher than those of the tocotrienol homologs. Among the tocopherol homologs, α-tocopherol was detected most often (in all 81 plants). Furthermore, 63.0%, 44.4%, and 35.8% of the plants contained the γ-, β-, and δ-tocopherol homologs, respectively. In all 81 plants, α- and β-tocotrienol homologs were found, while γ- and δ-tocotrienol homologs were not found. Figure 3 shows the combinations of homologs present in the plants. In total, 25.9% of the plants contained α-tocopherol alone, and the remaining 74.1% of plants contained more than two homologs. Overall, 18.5% of the plants contained a combination of all four tocopherol homologs; 17.3% of the plants contained α- and γ-tocopherol homologs; 14.8% contained α-, β-, and γ-tocopherol homologs; 4.9% contained α-, γ-, and δ-tocopherol homologs; 2.5% contained α-, γ-, and δ-tocopherol homologs with α-tocotrienol; and 1.2% continued four other combinations with γ-tocopherol. Thus, among the plants found to contain more than two homologs, 84.5% contained both α- and γ-tocopherol homologs.

The concentration of each homolog is given in Table 1. Among the 81 plants assessed, α-tocopherol was present in the highest concentration at 0.6-750.1 μg/g. Homologs with the next highest concentrations were β-tocopherol (0.6-33.3 μg/g), followed by γ-tocopherol (0.6-29.1 μg/g), α-tocotrienol (0.6-14.0 μg/g), δ-tocopherol (0.6-13.8 μg/g), and β-tocotrienol (0.8-5.3 μg/g).

Distribution of vitamin E homologs in different parts of the plants

The distribution of each homolog in different parts of the plants is shown in Table 2. The highest concentrations of vitamin E homologs were found in leaves, where six homologs were detected, including the four tocopherol homologs and α- and β-tocotrienol homologs. The next highest concentrations of tocopherol homologs were found in stems and flowers, where the four tocopherol homologs were

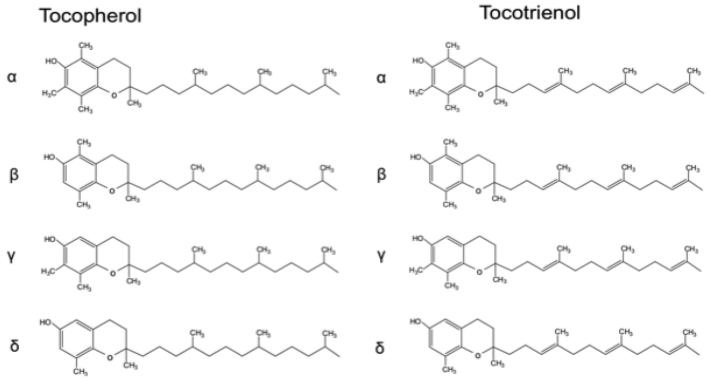

Figure 1: Chemical structures of tocopherol and tocotrienol.

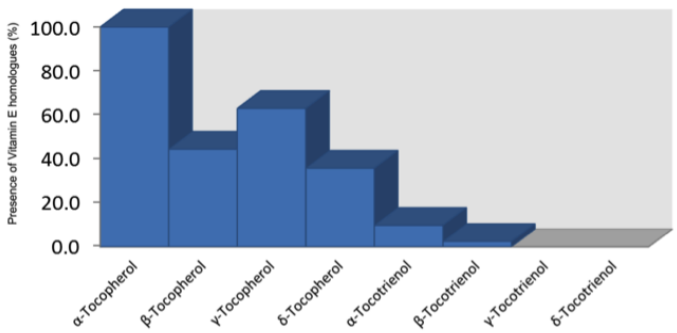

Figure 2: Distribution of tocopherols and tocotrienols in 81 plants. The presence of four tocopherols and four tocotrienols in 81 plants were analyzed using LC-MS3. Alpha-tocopherol was confirmed in all 81 plants, and γ- and δ-tocotrienols were not detected in any of the 81 plants.

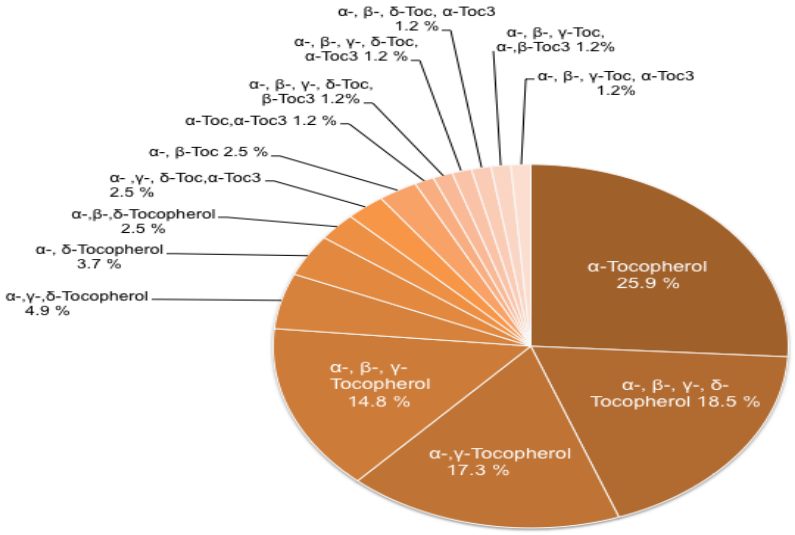

Figure 3: The distribution of vitamin E homologs in 81 plants. Only α-tocopherol was the most detected (25.9%), followed by α-, β-, γ- and δ-tocopherols (18.5%); α- and β-tocopherol (17.3%); α-, β-, and γ-tocopherols (14.8%); and α-, β-, and δ-tocopherol (4.9%). The presence of the combination of α-tocopherol and γ-tocopherol is remarkable.

Species	Tocopherol (μg/g dry tissue)				Tocotrienol (μg/g dry tissue)			
	α	β	γ	δ	α	β	γ	δ
Acmella oleracea (L.) R.K.Jansen	79.8 ± 0.2	0.6 ± 0.0	1.0 ± 0.0	nf	nf	nf	nf	nf
Adansonia digitata L.	8.0 ± 0.3	nf	nf	nf	nf	nf	nf	nf
Adansonia za Baill.	224.8 ± 0.3	1.0 ± 0.3	20.9 ± 0.1	0.6 ± 0.0	nf	nf	nf	nf
Adansonia za var. bosy	217.2 ± 5.8	6.9 ± 0.5	5.2 ± 0.1	2.0 ± 0.3	nf	nf	nf	nf
Agastache foeniculum (Pursh) Kuntze	72.1 ± 1.4	nf	2.0 ± 0.0	2.3 ± 0.0	nf	nf	nf	nf
Alluaudia procera Drake	0.7 ± 0.01	nf	nf	nf	nf	nf	nf	nf
Aloe vaombe Decorse & Poiss.	190 ± 7.4	1.3 ± 0.1	tr	7.3 ± 0.1	nf	nf	nf	nf
Ampelopsis japonica (Thunb.) Makino	148 ± 0.4	tr	0.6 ± 0.0	nf	nf	nf	nf	nf
Arctium lappa L.	48.2 ± 4.2	nf	0.6 ± 0.0	nf	nf	nf	nf	nf
Artemisia absinthium L.	35.4 ± 0.9	nf	3.8 ± 0.3	nf	nf	nf	nf	nf
Astragalus membranaceus (Fisch. ex Lnk) Bunge	57.1 ± 3.7	1.9 ± 0.0	2.5 ± 0.1	1.1 ± 0.0	nf	nf	nf	nf
Atractylodes ovata (Thunb.) DC.	108.0 ± 1.3	nf	nf	nf	nf	nf	nf	nf
Avicennia marina (Forssk.) Vierh.	6.3 ± 0.4	tr	tr	nf	nf	nf	nf	nf
Bruguiera gymnorrhiza (L.) Lam.	257.3 ± 6.3	nf	29.1 ± 0.2	1.1 ± 0.1	2.7 ± 0.0	nf	tr	nf
Bupleurum stenophyllum (Nakai) Kitag.	19.5 ± 2.3	nf	nf	nf	nf	nf	nf	nf
Catharanthus roseus G.Don	102.4 ± 1.0	3.4 ± 0.1	1.4 ± 0.3	3.9 ± 0.1	nf	nf	nf	nf
Celosia argentea L.	184 ± 2.0	1.6 ± 0.2	2.2 ± 0.1	0.9 ± 0.0	nf	nf	nf	nf
Ceratotheca triloba E.Mey. ex Bernh.	4.3 ± 0.2	0.6 ± 0.1	1.5 ± 0.0	nf	nf	nf	nf	nf
Chamaecrista nomame (Siebold) H.Ohashi	285.0 ± 0.9	nf	5.5 ± 0.2	nf	nf	nf	nf	nf
Cichorium intybus L.	7.8 ± 0.4	0.7 ± 0.1	9.0 ± 0.0	nf	nf	nf	nf	nf
Cnidium monnieri (L.) Cusson	84.6 ± 2.9	tr	tr	0.6 ± 0.0	0.6 ± 0.0	nf	nf	nf
Coix lacrymanfjobi L. var. manfyuen (Roman.) Stapf	8.0 ± 0.2	2.0 ± 0.1	4.7 ± 0.0	2.6 ± 0.0	nf	nf	nf	nf
Copaifera officinalis L.	200 ± 4.1	nf	1.6 ± 0.4	nf	nf	nf	nf	nf
Crateva religiosa G.Forst.	213.2 ± 2.6	10.6 ± 0.1	4.5 ± 0.1	1.1 ± 0.0	nf	nf	nf	nf
Didierea madagascariensis Baill.	213.8 ± 1.6	5.6 ± 0.1	7.2 ± 0.1	0.6 ± 0.1	nf	nf	nf	nf
Echinacea purpurea (L.) Moench	266.4 ± 4.3	1.3 ± 0.4	0.9 ± 0.0	nf	nf	nf	nf	nf
Elaeocarpus sylvestris var. ellipticus	0.6 ± 0.0	tr	tr	nf	0.6 ± 0.0	nf	nf	nf
Euphorbia stenoclada Baill.	8.4 ± 0.9	nf	nf	nf	nf	nf	nf	nf
Glycyrrhiza uralensis Fisch. ex DC.	21.8 ± 0.2	nf	1.0 ± 0.0	nf	nf	nf	nf	nf
Hibiscus sabdariffa L.	3.7 ± 0.0	nf	nf	nf	nf	nf	nf	nf
Hypoxidaceae Spiloxene Salisb.	23.9 ± 0.4	tr	tr	tr	nf	nf	nf	nf
Isodon japonicus (Burm.f.) H.Hara	113.2 ± 1.5	6.6 ± 0.1	10.3 ± 0.1	0.7 ± 0.0	nf	nf	nf	nf
Jatropha curcus L.	42.7 ± 1.4	nf	tr	nf	nf	nf	nf	nf
Kalanchoe beharensis Drake	72.9 ± 4.3	nf	nf	nf	nf	nf	nf	nf
Kalanchoe prolifera Raym.-Hamet	37.0 ± 3.9	0.7 ± 0.0	tr	0.6 ± 0.0	nf	nf	nf	nf

Kalanchoe synsepala Baker	26.1 ± 0.8	tr	1.9 ± 0.0	tr	nf	nf	nf	nf
Kalanchoe tubiflora Raym.-Hamet	68.4 ± 3.8	nf	nf	nf	nf	nf	nf	nf
Kandelia obovata Sheue , H.Y.Liu & J.W.H.Yong	0.8 ± 0.1	nf	1.1 ± 0.0	0.8 ± 0.1	0.9 ± 0.0	nf	nf	nf
Lavandula angustifolia Mill.	21.7 ± 0.2	6.5 ± 0.2	nf	nf	nf	nf	nf	nf
Leonurus japonicus Houtt.	33.5 ± 0.5	nf	2.1 ± 0.2	nf	nf	nf	nf	nf
Linum usitatissimum L.	156.6 ± 3.5	1.9 ± 0.5	4.5 ± 0.0	nf	nf	nf	nf	nf
Lycium chinense Mill.	57.7 ± 0.2	nf	7.5 ± 0.2	5.7 ± 1.6	nf	nf	nf	nf
Malva sylvestris L.	58.7 ± 4.1	1.0 ± 0.1	3.6 ± 0.1	0.6 ± 0.0	nf	nf	nf	nf
Malvaceae Hibiscus L.	2.1 ± 0.4	0.8 ± 0.2	0.6 ± 0.0	nf	nf	nf	nf	nf
Melaleuca alternifolia Cheel	18.0 ± 0.8	0.9 ± 0.03	0.6 ± 0.0	0.7 ± 0.0	nf	0.6 ± 0.0	nf	nf
Moringa drouhardii Jum.	125.3 ± 3.7	nf	nf	nf	nf	nf	nf	nf
Moringa oleifera Lam.	218.0 ± 0.8	1.1 ± 0.01	2.0 ± 0.1	nf	nf	nf	nf	nf
Myrtus communis L.	24.2 ± 1.3	nf	nf	0.6 ± 0.0	nf	nf	nf	nf
Neodypsis decaryii Jumelle	145 ± 1.6	nf	nf	nf	nf	nf	nf	nf
Operculicarya decaryii H.Perrier	750 ± 3.0	nf	6.3 ± 0.3	nf	nf	nf	nf	nf
Origanum majorana L.	5.5 ± 0.1	1.2 ± 0.1	2.2 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	nf	nf	nf
Origanum vulgare L.	2.3 ± 0.3	0.6 ± 0.1	1.1 ± 0.0	0.6 ± 0.0	nf	nf	nf	nf
Pachypodium lamerei var. ramosum	8.2 ± 3.0	0.6 ± 0.0	nf	2.2 ± 0.1	0.6 ± 0.0	nf	nf	nf
Pachypodium rutenbergianum var. meridionale	7.0 ± 0.8	1.3 ± 0.0	nf	nf	nf	nf	nf	nf
Pandanus dyckioides Baker	14.1 ± 0.2	tr	8.8 ± 0.1	tr	nf	nf	nf	nf
Patrinia scabiosifolia Fisch.ex Trevir	5.4 ± 0.4	tr	0.6 ± 0.0	0.7 ± 0.0	nf	nf	nf	nf
Perilla frutescens (L.) Britton var. frutescens	3.7 ± 0.2	nf	nf	nf	nf	nf	nf	nf
Persicaria tinctoria (Aiton) Spach	54.4 ± 4.3	1.4 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	nf	nf	nf	nf
Platycodon grandiflorus (Jacq.) A.DC.	43.4 ± 0.6	nf	2.0 ± 0.0	nf	nf	nf	nf	nf
Prunella vulgaris L. subsp. Vulgaris	268.4 ± 3.3	tr	0.8 ± 0.0	13.6 ± 0.1	nf	nf	nf	nf
Ravenea musicalis Beentje	468 ± 1.0	15.1 ± 0.5	3.3 ± 0.1	nf	14.0 ± 0.0	5.3 ± 0.7	nf	nf
Rhizophora mucronata Lam.	5.5 ± 0.3	2.5 ± 0.1	2.0 ± 0.0	tr	0.8 ± 0.1	nf	nf	nf
Rosmarinus officinalis L.	131.6 ± 2.6	3.1 ± 0.0	0.8 ± 0.0	tr	nf	nf	nf	nf
Salvia miltiorrhiza Bunge	259.5 ± 1.3	0.8 ± 0.2	1.0 ± 0.0	nf	nf	nf	nf	nf
Salvia officinalis L.	59.9 ± 3.3	nf	nf	nf	nf	nf	nf	nf
Sanguisorba minor Scop.	116.2 ± 0.3	nf	nf	3.6 ± 0.0	nf	nf	nf	nf
Saponaria officinalis L.	168.1 ± 1.5	1.7 ± 0.1	1.2 ± 0.0	nf	nf	nf	nf	nf
Scutellaria baicalensis Georgi	143.3 ± 1.3	tr	5.9 ± 0.2	nf	nf	nf	nf	nf
Simmondsia chinensis C.K.Schneid.	2.7 ± 0.1	6.0 ± 0.2	1.1 ± 0.1	nf	nf	nf	nf	nf
Sorghum bicolor (L.) Moench	25.1 ± 3.2	nf	nf	nf	nf	nf	nf	nf
Sporobolus virginicus Kunth	5.6 ± 0.2	nf	nf	nf	nf	nf	nf	nf
Symphytum officinale L.	30.3 ± 0.2	nf	nf	nf	nf	nf	nf	nf
Terminalia catappa Linn.	82.1 ± 0.4	1.7 ± 0.3	1.8 ± 0.2	tr	nf	nf	nf	nf
Tetragonia tetragonoides (Pall.) Kuntze	27.4 ± 2.6	1.4 ± 0.2	0.8 ± 0.0	0.7 ± 0.0	nf	nf	nf	nf
Tetrastigma lanceolarium Planch.	476 ± 1.4	nf	nf	nf	nf	nf	nf	nf
Thymus vulgaris L.	59.8 ± 1.2	0.9 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	nf	nf	nf	nf
Typhonodorum lindleyanum Schott	46.5 ± 0.7	nf	4.6 ± 0.2	nf	nf	nf	nf	nf
Uncarina grandidieri (Baill.) Stapf	4.8 ± 0.0	nf	nf	nf	nf	nf	nf	nf
Uncarina leandrii var. recbergii	83.2 ± 1.3	nf	nf	nf	nf	nf	nf	nf
Vanilla decaryana H.Perrier	2.5 ± 0.1	33.3 ± 1.4	1.1 ± 0.1	12.4 ± 0.0	nf	nf	nf	nf
Verbena officinalis L.	39.5 ± 0.3	tr	1.0 ± 0.0	nf	nf	nf	nf	nf

The amounts of eight vitamin E homologues are determined by the mean and standard deviation value of each vitamin E homologue in leaves, stems and branches of each plant. Values are the mean of three replicates ± SD.. nf, not found; tr, trace = detected but not quantified.

Table 1: The contents of tocopherols and tocotrienols in 81 kinds of plant.

Tissues	Tocopherol(μg/gdrytissue)				Tocotrienol(μg/gdrytissue)			
	α	β	γ	δ	α	β	γ	δ
Leaf	0.6 - 1470.4	0.3 - 15.1	0.6 - 41.7	0.4 - 13.1	0.6-14.0	0.6-5.3	nf	nf
Stem	0.3 - 446.0	0.8 - 33.3	0.2 - 23.4	0.3 - 20.6	nf	nf	nf	nf
Rod	38.3 - 47.5	7.2	3.0	nf	nf	nf	nf	nf
Flower	1.1 - 89.1	0.6 - 1.1	0.7 -7.9	1.3 - 4.7	nf	nf	nf	nf
Bud	26.0 - 72.8	nf	0.6 - 1.1	15.8	nf	nf	nf	nf
Ear	2.8 - 147.2	nf	nf	nf	0.6	nf	nf	nf

The amounts of eight vitamin E homologues are determined by the mean of each vitamin E homologue in different tissues of each plant. nf, not found; tr, trace = detected but not quantified.

Table 2: The contents of tocopherols and tocotrienols in different tissues in 81 kinds of plant.

identified. Branches and buds of the plants contained the next highest concentrations of three tocopherol homologs. In contrast, ears only contained α-tocopherol.

When homolog distributions were examined, α-tocopherol was present in all parts of the plants, whereas β-tocopherol was present in leaves, stems, flowers, and buds. Moreover, γ-tocopherol was present in leaves, stems, flowers, branches, and buds, whereas δ-tocopherol was present in leaves, stems, flowers, and buds. Tocotrienol homologs were only found in leaves and not in any other parts of the plants.

The homolog concentrations in leaves, where most homologs were found, are shown in Table 3 with respect to the plant family. A particularly high concentration of homologs was found in leaves of the sumac and mint families, where concentrations of α-tocopherol were 1470.4 μg/g and 699.1 μg/g, respectively. Only 0.6 μg/g each of α-tocopherol and α-tocotrienol was detected in Elaeocarpaceae plants.

Discussion

This is the first study to use LC-MS3 for analyzing the eight vitamin E homologs and clarifying their distributions in plants. It was found that tocopherol homologs, which exhibit a stronger antioxidant activity than tocotrienol homologs [10,11], exist at higher concentrations in plants than tocotrienol homologs. Among the eight homologs, α-tocopherol having the highest antioxidant activity [10,11] was present at high concentration. The concentrations of β, γ and δ-tocopherol in plants tended to depend on the strength of each antioxidant activity. In the tocotrienol homologs with low antioxidant activity [10,11], α and β forms were detected slightly and γ and δ forms were not detected. From these results, the distribution of vitamin E homologues may be related to the antioxidant activity of each homolog. Therefore, the distribution of vitamin E homologs may be related to the intensity of the antioxidant activity of each homolog. α-Tocopherol, which showed a high antioxidant activity, was present in leaves of plants belonging to

Family	Tocopherol (μg/g dry tissue)				Tocotrienol (μg/g dry tissue)			
	α	β	γ	δ	α	β	γ	δ
Acanthaceae	6.3	tr	tr	nf	nf	nf	nf	nf
Aizoaceae	12.3	2.5	0.6	2.1	nf	nf	nf	nf
Aloaceae	190.1	1.3	tr	7.3	nf	nf	nf	nf
Amaranthaceae	484.8	4.7	3.8	tr	nf	nf	nf	nf
Anacardiaceae	1470.4	nf	8.3	nf	nf	nf	nf	nf
Apiaceae	22.1 - 70.5	tr	tr	0.9	nf	nf	nf	nf
Apocynaceae	7.0 - 26.0	0.9 - 1.3	nf	3.7 - 6.0	0.8	nf	nf	nf
Araceae	73.8	nf	4.4	tr	nf	nf	nf	nf
Arecaceae	144.6 - 467.5	15.1	3.3	nf	14.0	5.3	nf	nf
Asteraceae	3.0 - 519.2	2.1 - 2.6	1.6 - 11.3	nf	nf	nf	nf	nf
Bombacaceae	3.5 - 106.9	2.0 - 11.8	0.7 - 41.7	3.4	nf	nf	nf	nf
Boraginaceae	57.7	tr	tr	tr	nf	nf	nf	nf
Buxaceae	5.1	12.0	0.6	nf	nf	nf	nf	nf
Caesalpiniaceae	541.7	tr	11.0	nf	nf	nf	nf	nf
Campanulaceae	75.9	tr	4.0	tr	nf	nf	nf	nf
Capparaceae	213.2	10.6	4.5	1.1	nf	nf	nf	nf
Caryophyllaceae	259.1	3.3	2.3	nf	nf	nf	nf	nf
Combretaceae	82.1	1.7	1.8	tr	nf	nf	nf	nf
Crassulaceae	5.6 - 144.5	1.3	1.9	nf	nf	nf	nf	nf
Didiereaceae	0.7 - 213.8	5.6	7.2	0.6	nf	nf	nf	nf
Elaeocarpaceae	0.6	tr	tr	nf	0.6	nf	nf	nf
Euphorbiaceae	8.4 - 98.9	1.8	2.3	nf	nf	nf	nf	nf
Fabaceae	42.9 - 200	nf	1.6 - 1.9	nf	nf	nf	nf	nf
Hypoxidaceae	23.9	tr	tr	tr	nf	nf	nf	nf
Lamiaceae	0.8 - 699.1	0.6 - 10.9	1.0 - 18.3	0.6 - 4.5	0.9	nf	nf	nf
Linaceae	297.8	tr	3.0	tr	nf	nf	nf	nf
Malvaceae	2.8 - 71.3	1.5	1.1 - 2.0	nf	nf	nf	nf	nf
Moringaceae	97.0 - 242.5	2.1	3.9	nf	nf	nf	nf	nf
Myrtaceae	14.5 - 19.6	1.8	1.1	0.7 - 1.4	nf	0.6	nf	nf
Pandanaceae	14.1	tr	8.8	tr	nf	nf	nf	nf
Pedaliaceae	2.2 - 141.8	tr	0.9	nf	nf	nf	nf	nf
Poaceae	5.6 - 57.2	nf	2.6	nf	nf	nf	nf	nf
Polygonaceae	83.4	0.7	0.9	0.6	nf	nf	nf	nf
Rhizophoraceae	0.8 - 257.3	2.5	1.1 - 29.1	0.8 - 1.1	0.8 - 2.7	nf	nf	nf
Rosaceae	172.8	tr	tr	7.1	nf	nf	nf	nf
Solanaceae	90.4	tr	14.5	13.1	nf	nf	nf	nf
Valerianaceae	1.5	nf	nf	0.8	nf	nf	nf	nf
Verbenaceae	36.9	tr	tr	nf	nf	nf	nf	nf
Vitaceae	170.8 - 475.5	nf	nf	nf	nf	nf	nf	nf

The amounts of eight vitamin E homologues are determined by the mean of each vitamin E homologue in leaves of each plant. nf, not found; tr, trace=detected but not quantified.

Table 3: The contents of tocopherols and tocotrienols in different dry tissues.

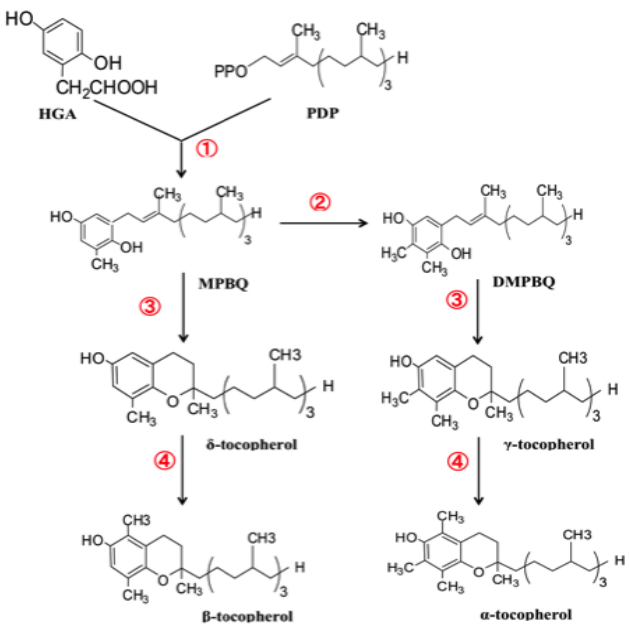

Figure 4: The tocopherol biosynthetic pathway in plants. Compound abbreviations: HGA, homogentisic acid; PDP, phytyl diphosphate; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone. Circled numbers refer to the enzymes: (1) Homogentisic acid phytyl transferase. (2) 2,3-dimethyl-5-phytyl-1,4-benzoquinone transferase. (3) tocopherol cyclase. (4) γ-tocopherol methyl transferase.

the sumac family, which is native to tropical regions. Active oxygens such as super oxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are produced after exposure to high light intensity [12-15]. Because tropical plants are highly exposed to environmental stresses such as high temperature and high light intensity, active oxygens are readily produced in tropical plants. This suggests that α-tocopherol, which possesses the highest active oxygen scavenging activity, is present at a high concentration in plants to support plant life [11].

Lipid-soluble tocopherol homologs are present in plant parts where there is a large amount of lipids present [16,17]. The presence of tocopherol homologs has been reported in rice bran [3], seed, endosperm [4], barley, wheat, and rice [5]. The current study revealed that α-, β-, γ-, and δ-tocopherol homologs were also present in leaves and stems. Leaves and stems contain chloroplasts, where lipid-containing thylakoid membranes are found [18-20]. This may explain the high levels of lipid-soluble α-tocopherol homologs that are present in leaves and stems. Thus, the distribution of tocopherol homologs may be influenced by the lipid solubility of tocopherol homologs.

Simultaneous analysis of the eight homologs revealed their distribution patterns for the first time. Furthermore, we revealed that α-tocopherol in combination with γ-tocopherol most often found in plants. As shown in Figure 4, α-tocopherol is synthesized from homogentisic acid via 2,3-dimethyl-5-phytyl-1,4-benzoquinone and γ-tocopherol [21-23]. In other words, γ-tocopherol is a precursor of α-tocopherol, which is the homolog most often found in plants. Thus, the presence of α-tocopherol in combination with γ-tocopherol may be because of the α-tocopherol synthetic pathway in plants.

Conclusions

This study elucidated the distribution of the eight vitamin E homologs by analyzing vitamin E homologs in plants using LC-MS3. The results indicated that the distribution of vitamin E homologs was affected by the intensity of the antioxidant activity. Alpha-tocopherol, which showed the highest antioxidant activity, may be present in all plants to support plant life by acting as an active oxygen scavenger. The common occurrence of α-tocopherol in combination with γ-tocopherol in plants was predicted to be because of the α-tocopherol synthetic pathway. The highest distribution of α-tocopherol homologs was found in the plant leaves. This finding indicates that lipid-soluble tocopherol homologs are localized to the lipid component of leaf chloroplasts. Thus, the distribution of vitamin E homologs was found to be related to supporting plant life.

From this study, it was clarified that ingestion of vitamin E in plants can efficiently ingest tocopherol homologs with high antioxidant activity including the α form. This study can provide more knowledge about the nutritional value of vitamin E in plants.

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